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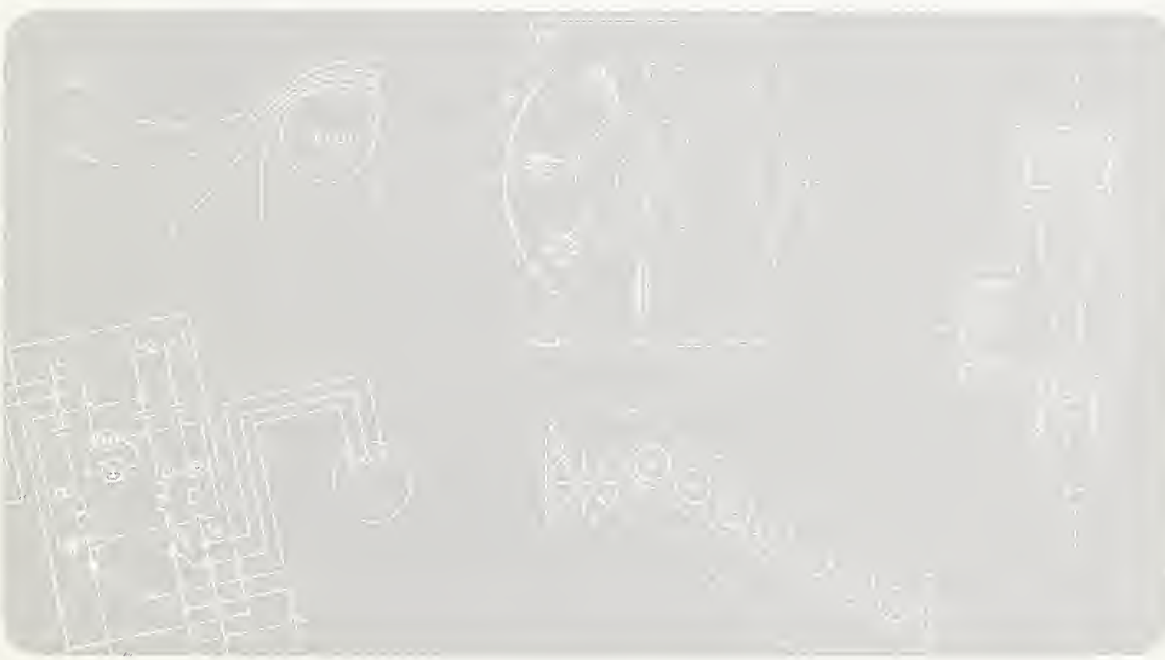
Detection of Hidden Insect Infestations in Wheat by Infrared Carbon Dioxide Gas Analysis



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Detection of Hidden Insect Infestations in Wheat by Infrared Carbon Dioxide Gas Analysis

By William A. Bruce,[1] M. Wayne Street,[2] Randall C. Semper,[3] and David Fulk[4]

ABSTRACT

A method for the detection of hidden insects by means of infrared CO_2 gas analysis that measures insect-produced CO_2 given off during respiration was tested under limited field conditions. The 2-year study has demonstrated that this method is significantly better than present routine inspection procedures at detecting hidden insects. Of the 459 samples tested 96% were eventually found to be infested. Only 11% of the infestations were detected with current procedures; but IR CO_2 analysis detected 81%. Index terms: grain insects, grain inspection, insect detection, inspection (agricultural products), wheat insects, wheat inspection.

INTRODUCTION

The need to detect hidden insect infestations in agricultural commodities throughout the marketing channel has long been recognized by consumers, regulatory agencies, research scientists, and administrators. But destruction and damage caused by insects to food and food ingredients have continued to increase. Growing pressure for increased efficiency of yield and protection from insect damage have intensified the need for detecting this kind of infestation. Early detection of hidden insects is also important in agricultural inspections made for quality control, quarantine, and other purposes. Approaches to detection have embodied several physical, chemical, and biological principles (see, for example, Adams et al. 1954, Milner 1958, Dennis and Decker 1962, Bruce et al. 1978, and Bruce and Street 1979). Detection techniques can be either active or passive. Active techniques require that something be added to the substance being tested. Passive techniques depend only on naturally occurring outputs such as infrared energy radiated by the insects, sounds produced by feeding, or gas produced by insect metabolism or respiration (Bruce and Street 1974).

Currently, one method being investigated for insect detection purposes is infrared (IR) CO_2 analysis. This system has been described in detail previously from the physical (Bruce and Street 1974, Street and Bruce 1976b) and biological (Bruce and Street 1975, Street and Bruce 1976a) aspects. The advantages of this system over other methods are speed, reliability, repeatability, and sensitivity.

To determine how effective IR CO_2 gas analysis is in detecting hidden insect

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infestations from field-collected samples of wheat, a cooperative test involving the U.S. Federal Grain Inspection Service (FGIS) and U.S. Agricultural Research Service (ARS) was established. The objectives of this study were to: evaluate the system's effectiveness in detecting hidden insect infestations, determine if dockage alters the effectiveness of the detection system, and determine if IR CO_2 analysis systems placed in Houston, Tex., and Savannah, Ga., would yield similar results.

OPERATION OF THE DETECTION SYSTEM

Operation of the insect-detection system (fig. 1) may be conveniently divided into four steps: prepurge, system purge, sample collection, and sample readout.

Prepurge

The prepurge is used to flush accumulated gases out of a sample-filled test chamber just before its connection to the detection apparatus. If a sample of unknown quality has been left unattended for any length of time, it may contain entrained CO_2 from insects in the sample or from slow respiration of the sample itself or of fungi, particularly at elevated moisture contents. So the prepurge simply flushes out any accumulated CO_2 and reduces the time required for online equilibration during the next step, system purge. In actual practice, prepurging can be arranged so that, as each sample is being tested, the next oncoming sample is being prepurged.

System Purge

The system uses an interrupted-flow principle to detect deviation from an equilibrium baseline. This baseline is established during system purge. Immediately after the test chamber is connected to the system, the carrier gas is allowed to flow until the differential output from the CO_2 analyzer stabilizes, indicating equilibrium, which is generally zero except when infestations are heavy (Street and Bruce 1976b).

Sample Collection

During sample collection, carrier gas flow is cut off and the analyzer and sample system sealed by inline valves to permit buildup of the insect-produced CO_2 in the test chamber. At the onset of the sample-collection interval, the reference cell, test chamber, and sample cell contain equal concentrations of CO_2 because equilibrium has been reached during the preceding system purge. As soon as carrier gas flow ceases, the following occur: In the reference cell, the nominal CO_2 concentration remains essentially constant since the carrier gas is trapped and nonflowing; in the test chamber, the CO_2 concentration begins to rise above the equilibrium value because of the addition of the insect-produced CO_2; in the sample cell, a condition similar to that in the reference cell exists—the trapped carrier gas is nonflowing and has the equilibrium concentration of CO_2 reached during system purge. Since there is no flow, any diffusion of CO_2 beyond the boundaries of the test chamber should occur in both directions and, because of system symmetry, produce minimal differential imbalance between the analyzer reference and sample cells. No discernible imbalance caused by CO_2 diffusion has been observed in the prototype system. But, should it occur, it could be eliminated simply by adding valves on each side of the test chamber. At the end of the sample-collection interval, the test chamber will contain a concentration of CO_2 that is higher than that in the analyzer reference and sample cells.

Sample Readout

The valves are opened to unseal the system, and the airflow is restarted. The higher concentration bolus of CO_2 moves out of the test chamber and through the sample cell of the IR analyzer. The resulting imbalance between the CO_2 concentration in the two optical cells of the IR analyzer generates a signal that is displayed as a peak on the chart recorder. After the bolus has passed through the system, equilibrium is again reached where conditions are stable among the reference cell, test chamber, and sample cell. As soon as equilibrium is reached,

sample collection and sample readout may be repeated as often as desired.

MATERIALS AND METHODS

Detection Equipment

Houston

The equipment for the insect-detection system as described consists basically of an IR CO_2 analyzer, a series of solenoid valves and timer to control airflow, a small pump, a test chamber, and a signal readout device (meter or strip-chart recorder). The systems constructed and supplied by ARS to FGIS, for evaluation, were two portable, self-contained laboratory units for use at the port of Houston, Tex. Each unit consisted of a Mine Safety Appliances (MSA; see appendix for a list of supplier names and addresses) Luft-type differential IR CO_2 analyzer with a sensitivity of 0–100 p/m (parts per million) CO_2, two Industrial Timer Corp. collect-readout timers, two Dwyer airflow meters, a Universal Electric Co. 1.5-amp pump, an E&K 8-inch strip-chart recorder, and quick-disconnect ports for test chamber hookup. Technicon Instruments Corp. placed a detection system in Houston that was similar to this configuration but different in several features. Their system used a Horiba Instruments PIR-2000 IR CO_2 gas analyzer with a high sensitivity range of 0–25 p/m CO_2. A total of three test chambers allowed samples to be analyzed sequentially and simultaneously

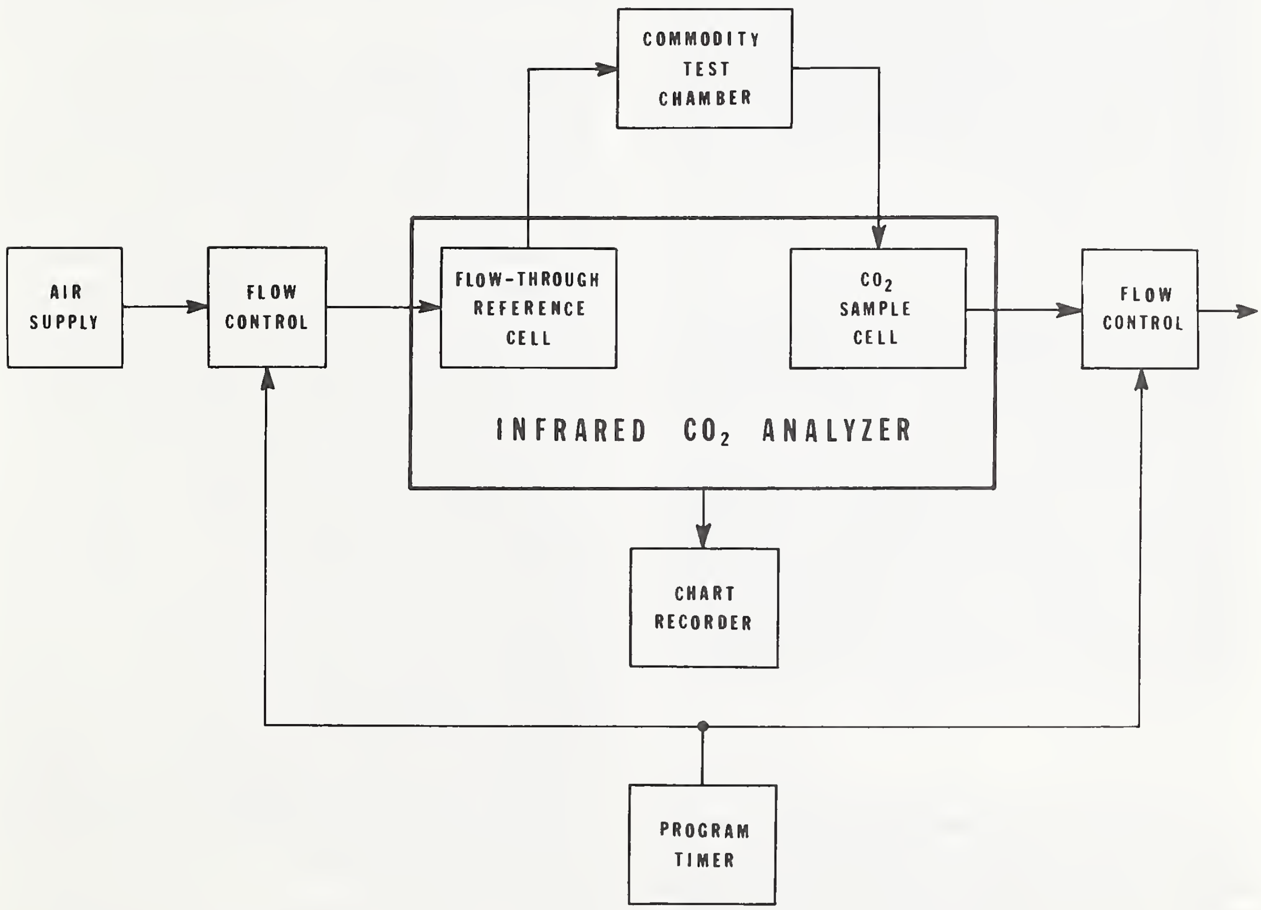

Figure 1.—Components of insect-detection system. (Source: Street and Bruce 1976b.)

through prepurge, CO_2-sample collection, and CO_2-sample readout. A gas (CO_2) calibration unit was incorporated to insure accurate and rapid calibration of the system.

Savannah

The Savannah system used a Beckman Instruments Model 865 and a Horiba PIR-2000 IR CO_2 gas analyzer. All remaining components were essentially the same as the Houston MSA system except that they were not installed in a cabinet but were arranged on a bench top to facilitate operating and research procedures.

Test Procedure

The test procedure of the insect-detection system was designed by R. C. Semper. The tests were conducted at the FGIS Houston field office, and at the ARS Stored-Product Insects Research and Development Laboratory in Savannah. The test procedure had three stages: sample collection of hard red winter wheat at Houston, testing at the Houston field office, and testing at Savannah.

Insect Rearing

Each wheat sample consisted of four subsamples: A, B, A dockage, and B dockage. After the subsamples were analyzed for CO_2, each was placed in a quart jar and then taken to a holding room maintained at about 27° C and 80% relative humidity for at least 9 weeks. Care was taken to insure that no insect contamination occurred during the handling process. After the appropriate rearing period had elapsed, each sample was again observed for insects, alive or dead, analyzed for CO_2, and X-rayed when required. Then, all samples were discarded.

X-ray Analysis

A Hewlett-Packard Faxitron model 43804N was used for X-ray inspection. Exposure time and operating voltage can be varied for optimum results. The wheat for X-ray was first spread in a monolayer directly onto Kodak Radioprint TP6 paper and then placed into the X-ray unit at 30 kV for 60 seconds. After exposure to X-rays, the Radioprint TP6 paper was developed in a Kodak Ektachrome automatic processor. The radiograph was visually inspected for the presence of insects. Samples designated for X-ray analysis were those that had a positive CO_2 reading but had no adult insects emerge after the 9-week rearing period. Samples without a positive CO_2 reading and with no adult emergence were X-rayed to check for extremely low levels of infestation or infestations of insects that may have died before sample collection..

RESULTS

Standard Versus New Test Procedures

The results obtained using IR CO_2 analysis for detection of hidden insect infestations indicate the potential for use of this method in routine grain inspection. Using current FGIS standard procedures to determine the presence of insects in a particular sample gave the following results: (1) 6 infestations were noted visually in 597 samples (1.0%) at the grain-loading facility (of 600 samples, Nos. 22, 23, and 126 were missing); (2) 59 more infestations were noted visually by Houston personnel in the 597 samples (10%). Therefore, the procedures normally used by FGIS personnel to determine the presence of at least 2 live adult insects per sample accounted for 65 of 597 total samples or 11%. The testing done at Houston and Savannah showed that, of 459 samples received (Nos. 142–600), 440 (96%) were infested. This percentage includes all insect life stages, and results are derived from visual, IR CO_2, rearing, and X-ray analysis of samples. Current FGIS standards are restricted to identifying only live adults before a sample can be defined as weevily. But, while the methods studied include procedures not allowable under current inspection routines, these data show that rates of insect infestation in U.S. hard red winter wheat processed for export through the port of Houston during the study period were considerably higher than reported.

Equipment Performance

The Beckman 865 CO_2 analyzer at Savannah malfunctioned during analysis of samples

Table 1.—IR CO_2 analysis of hard red winter wheat for hidden insect infestations

Location	IR CO_2 detection system	Total No. samples	Samples indicated positive: Total No.	Emerged	X-ray +	X-ray –	X-ray ?	Samples not indicated positive: Total No.	Emerged	X-ray +	X-ray –	X-ray ?
Houston ...	MSA	459	[1]146	127	13	..	..	[2]313	237	63	1	3
Houston ...	Technicon Horiba.	228	193	171	22	..	..	[3]35	32	2	..	..
Savannah..	Horiba	459	373	316	52	3	2	[2]86	21	51	2	3

[1]2 samples positive, nothing found, no X-ray.
[2]9 samples not positive, nothing emerged, no X-ray.
[3]1 sample not positive, nothing found, no X-ray.

1-141, so only samples 142-600 were used for comparative purposes unless otherwise noted. These samples were inspected with the Horiba PIR-2000 IR CO_2 analyzer. Because its sensitivity (0-25 p/m) was four times that of the MSA units in Houston, the Horiba was used as the standard.

From all indications, the equipment, including IR CO_2 analyzers (except Beckman 865), timers, solenoid valves, flowmeters, etc., performed extremely well. Most problems that did arise involved dirty IR and air filters, analyzer calibration, and other conditions that could be resolved with normal maintenance procedures. FGIS and ARS personnel reported that they experienced no difficulty with the system operation and inspection procedure, though occasionally a question would arise concerning analyzer stability or electrical background noise levels and the need for analyzer calibration.

Because of better range of sensitivity, it was anticipated that the systems using Horiba analyzers would detect more hidden insect infestations. Comparison of the results (table 1) of the three systems shows that this was so. Although the number of samples analyzed with the Houston Technicon (Horiba) was about one-half that analyzed by the Savannah Horiba, the results were very similar. The Houston Technicon system gave a positive indication of insects 85% of the time and the Savannah Horiba system 81%. Actual emergence from these hidden, positive infestations was 89% (Houston Technicon) and 85% (Savannah Horiba). But the Houston MSA system indicated a positive infestation only 32% of the time and gave no indication of infestation 68% of the time. And, of the 313 samples not indicated as infested, 76% eventually produced an infestation.

IR CO_2 Analysis

The IR CO_2 analysis of grain samples used three different system configurations (Houston MSA, Houston Technicon (Horiba), Savannah Horiba) and four different analyzers. Savannah experienced problems with electrical noise early in the test with the Beckman 865; Houston MSA instrument No. 1 had some slight electrical noise throughout the test and at one point was reported to have excessive electrical noise probably caused by a dirty IR filter. Therefore, all data used from Houston MSA are based on instrument No. 2. Houston Technicon, brought online at sample No. 347, experienced some minor problems that were corrected by Technicon personnel. During the repair period, 26 samples were inspected only with the Houston MSA equipment.

IR CO_2 analysis data (Savannah Horiba) show that, of 459 samples (Nos. 142-600) tested, 373 or 81% were suspect (positive or suspected infestation); after the 9-week rearing period an infestation was observed visually in 316 of these samples (table 1). X-ray analysis revealed that 52 (91%) of the 57 suspect samples that had no emergence after the rearing period were infested, 2 were suspect, and 3 were negative.

X-ray Analysis

A total of 503 subsamples (dockage A, B; wheat A, B) representing 217 samples were subjected to X-ray analysis (table 2). About 4,000 radiographs were analyzed to see if they supported the findings of IR CO_2 analysis. Analysis of the dockage material was particularly difficult and time consuming because there were differences in the kinds of seeds present

Table 2.—X-ray analysis of subsamples

Subsample type	Total No.	Infestation		
		Positive	Suspected	Negative
Dockage ...	110	27	45	38
Wheat kernels.	393	393	....	....

Table 3.—Visual inspection of samples after 9-week rearing period

Sample Nos.[1]	Total	Infested	Emerged	
			Live	Dead
1-141	138	122	43	79
142-600	459	337	257	80
1-600	597	459	300	159

[1]Sample Nos. 22, 23, and 126 were missing.

Table 4.—Insect species[1] identified in wheat samples

Month and year	Total samples per month	Number of wheat samples infested with—							
		Rice weevil	Lesser grain borer	Flat grain beetle	Saw-toothed grain beetle	Moth	Weevil	Grain beetle	Other[2]
1977									
October	19	5	11	1	....	7	7	2	2
November[3]	31	9	10	1	1	11	15	1	12
December	46	23	15	....	1	14	2	....	5
1978									
January[4]	30	20	20	9	....	7	....	....	2
February	26	18	11	1	....	3	....	....	....
March	10	6	1	....	....	3	....	1	1
April	75	37	16	3	1	4	17	3	1
May	41	22	14	....	....	9	9	3	....
June	60	22	17	....	4	5	8	4	6
July	30	8	12	....	....	9	7	1	8
August	36	17	16	....	4	9	4	....	9
September	35	14	23	10	....	5	6	2	8
October	47	25	37	11	3	13	9	2	9
November	23	9	16	6	2	10	4	2	1
December	11	3	6	3	4	9	2	....	5
1979[5]									
March	58	24	25	3	....	2	12	3	14
April	19	9	5	2	....	1	6	3	5
Total	597	271	255	50	20	121	108	27	88

[1]Rice weevil, *Sitophilus oryzae* (Linnaeus); lesser grain borer, *Rhyzopertha dominica* (Fabricius); flat grain beetle, *Cryptolestes pusillus* (Schönherr); sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus). Moths were Indian meal moth, *Plodia interpunctella* (Hübner); Angoumois grain moth, *Sitotroga cerealella* (Olivier); and Mediterranean flour moth, *Anagasta kuehniella* (Zeller); moths usually could not be identified because of their fragmented condition. Weevils were granary weevil, *Sitophilus granarius* (Linnaeus), and maize weevil, *Sitophilus zeamais* Motschulsky; weevils usually could not be identified because of their fragmented condition. Grain beetles were merchant grain beetle, *Oryzaephilus mercator* (Fauvel); squarenecked grain beetle, *Cathartus quadricollis* (Guérin-Méneville); and foreign grain beetle, *Ahasverus advena* (Waltl).

[2]Includes black carpet beetle, *Attagenus megatoma* (Fabricius); cigarette beetle, *Lasioderma serricorne* (Fabricius); confused flour beetle, *Tribolium confusum* Jacquelin du Val; red flour beetle, *Tribolium castaneum* (Herbst); corn sap beetle, *Carpophilus dimidiatus* (Fabricius); mites, *Tyrophagus* spp. and *Acarus* sp.; and unidentifiable insect parts.

[3]No. 126 was missing.

[4]Nos. 22 and 23 were missing.

[5]No samples taken in January 1979 or February 1979 because of lack of technician help.

and in how much X-rays penetrated each. Analysis of radiographs of wheat kernels was equally as time consuming but more definitive.

Insect Rearing

Results of the visual inspection of samples after the 9-week rearing period revealed that 77% of the 597 samples had evidence of emergence (adult insects) and were therefore considered infested (table 3). Examination of samples 1-141 revealed 79 samples that had been infested, but all emerged adults were dead. The high percentage (65%) of such samples probably resulted because lack of enough technicians caused many samples to be inspected several months after the scheduled time; so the insects had starved after eating all available food.

More than 10 insect species and 1 mite species were identified (table 4). Samples were collected from October 1977 through April 1979; only in 1978 are data available for all 12 months of the year. But, because of equipment and support problems in March 1978 and December 1978, relatively few samples were received for those months.

DISCUSSION

The results indicate that IR CO_2 analysis and the instrumentation tested are significantly better than present FGIS standard procedures at detecting hidden insect infestations. This better performance is understandable since IR CO_2 analysis is able to detect CO_2 emitted both from individual insects that have emerged from the kernels of grain and from those that are concealed and developing in the kernel. Of course, it would be difficult for IR CO_2 analysis to detect, for example, one insect egg or one sawtoothed grain beetle in a 1,000-g grain sample in the time allowed by current inspection procedures. But such pinpoint detection would be possible if the inspection procedures were changed to allow the extra time needed (Street and Bruce 1976b). Even if the baseline of CO_2 sensitivity were raised to correspond only to levels of CO_2 considerably higher than those produced by one insect (for example, CO_2 produced by 50 rice weevils in 5 minutes), the detection capability would still be superior to current standards. And decreasing the sensitivity would increase the ratio of signal to noise (electrical), which would in turn increase the overall stability of the system.

The second objective of the study was to determine if dockage would alter the effectiveness of the detection system. There was no indication that dockage would interfere with the detection capability of the system. But frequent or excessive dockage would require a more frequent change of inline dust filters. And removal of dockage to be inspected separately did cause some concern at the Savannah laboratory. Because the volume of dockage present in each sample may vary, the dockage-sample container should be able to conform to these differences to eliminate excessive interstitial airspace. This potential problem was apparently corrected by putting the dockage in a Nitex bag and placing it in a standard test chamber filled with an inert material (glass beads). So the wheat sample and dockage were inspected in the same test chamber with essentially the same interstitial air volume. Of course, in a normal inspection routine, the dockage would not be separated but would be inspected with the wheat kernels.

The third objective of the study was to determine if the results from detection units would be about the same. Results with similar analyzers were, in fact, similar overall. Other than MSA analyzer No. 1 having had, on occasion, slightly more electrical background noise than MSA No. 2, both performed equally well. The two Horiba analyzers performed similarly overall but did not always give the same result for a particular sample. That samples were inspected first in Houston and then several days later in Savannah also made interpretation and comparison of data somewhat difficult. For instance, a given population would be older when it arrived in Savannah and might therefore be expected to have a higher output of CO_2. But more individuals may have reached the pupal stage, a factor that would tend to lower the CO_2 output. Or the population may have been alive in Houston but dead when the sample reached Savannah, and subsequent inspection might detect a limited quantity of residual or entrained insect-produced CO_2 still in the kernel. Also, the population could have died sometime during the Savannah rearing period. These possibilities may explain those instances

where infestations were detected in samples having no living insects (table 1).

The X-ray radiographs taken of samples after the 9-week rearing period supported the effectiveness of IR CO_2 analysis. Interpretation of the radiographs was sometimes difficult. But they did indicate that 91% of the nonpositive (Savannah Horiba), nonemerged samples that were X-rayed had been infested, though the insects had died. Both Technicon Horiba and Savannah Horiba detected live infestations in more than 80% of the samples. And, though further analysis (rearing and X-ray) showed that most of the nonpositive samples had, in fact, been infested, IR CO_2 analysis is far more effective than current routine inspection procedures. And it is far less costly and time consuming than insect rearing and X-ray analysis.

As the world demand for quality food, feed, seed, and fiber increases, the need to insure this quality against insect attack becomes even more important. The results of this study indicate the potential of IR CO_2 analysis to detect hidden insect infestations throughout the marketing channel. Clearly, this system could be successfully incorporated into an FGIS inspection routine if appropriate changes were made in the current inspection standards (for example, inclusion of immature stages of insects). Present studies also suggest that the greatest potential of the system may be to monitor for insects in the vast bulk storage of grain held throughout the United States, therefore helping maintain grain quality for both domestic and foreign markets.

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APPENDIX.—SUPPLIER NAMES AND ADDRESSES

Beckman Instruments
Fullerton, Calif.

Dwyer
Michigan City, Ind.

E&K Scientific Products
Saratoga, Calif.

Hewlett-Packard
McMinnville, Oreg.

Horiba Instruments
Irvine, Calif.

Industrial Timer Corp.
Parsippany, N.J.

Kodak—Eastman Kodak
Rochester, N.Y.

Mine Safety Appliances
Pittsburgh, Pa.

Nitex—Tobler, Ernst, and Traber
Elmsford, N.Y.

Technicon Instruments Corp.
Tarrytown, N.Y.

Universal Electric Co.
Owosso, Mich.

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